Enhancement of Human Immunodeficiency Virus Type 1–Specific CD4 and CD8 T Cell Responses in Chronically Infected Persons after Temporary Treatment Interruption

Emmanouil Papasavvas,1,4 Gabriel M. Ortiz,5,a Robert Gross,1,2,3 Junwei Sun,1 E. Caroline Moore,1 Jonas J. Heymann,5 Mona Moonis,1 Johan K. Sandberg,5 Lea Ann Drohan,5 Barbara Gallagher,4 Jane Shull,4 Douglas F. Nixon,5 Jay R. Kostman,1,4 and Luis J. Montaner1

Immunologic and virologic outcomes of treatment interruption were compared for 5 chronically human immunodeficiency virus (HIV)-infected persons who have maintained antiretroviral therapy–mediated virus suppression, as compared with 5 untreated controls. After a median interruption of 55 days of therapy accompanied by rebound of virus, reinitiated therapy in 4 of 5 subjects resulted in suppression of 98.86% of plasma virus load by 21–33 days and no significant decrease in CD4 T cell percentage from baseline. Increased T helper responses against HIV-1 p24 antigen (P = .014) and interferon-γ–secreting CD8 T cell responses against HIV-1 Env (P = .004) were present during interruption of therapy and after reinitiation of treatment. The remaining subject whose treatment was interrupted did not resume treatment and continued to have a low virus load (<1080 HIV-1 RNA copies/mL) and persistent antiviral cell-mediated responses. In summary, cellular immunity against autologous HIV-1 has the potential to be acutely augmented in association with temporary treatment interruption in chronically infected persons.

Treatment guidelines for human immunodeficiency virus type 1 (HIV-1) infection center on achieving virus suppression by use of highly active antiretroviral therapy (HAART), on the basis of the association between virus suppression and improved clinical outcome [1–6]. The current goal of HAART is to sustain lifelong suppression without treatment interruption with the hope for eradication of the virus if therapy is sustained. However, the feasibility of lifelong HAART-mediated virus suppression is limited by adverse drug effects, treatment cost, and the difficulty of maintaining optimal adherence for a prolonged duration [7, 8]. Moreover, the recent discovery of a long-lived latent reservoir of HIV-1 and a low level of viral replication in spite of undetectable plasma viremia has raised concern about the feasibility of virus eradication with the use of antiretroviral regimens [9, 10].

The potential role of cellular immune responses to control HIV-1 replication has been raised in light of data from studies of acute infection showing that virus-specific cytotoxic T lymphocytes are associated with a decrease in plasma viremia [11]. High-level anti-HIV–specific immune responses are associated with low viral replication, delay in the progression to AIDS in chronically infected persons [12–16], and protection from infection in high-risk exposed persons [17, 18]. HIV-1–infected long-term nonprogressors maintain high CD4 T cell proliferative responses to p24 antigen and to recall antigens, as well as high levels of HIV-1–specific CD8 T cell responses in association with low virus loads [13]. Unfortunately, the vast majority of HIV-1–infected persons fail to suppress plasma virus below 40 copies/mL in the absence of therapy and lose the T cell proliferative responses soon after infection [19, 20].

Antiretroviral therapy has many beneficial effects in chronically infected persons. Irrespective of the disease stage during which it is started, antiretroviral-mediated suppression can facilitate restoration of CD4 T cell proliferative responses to recall antigens and can fill CD4 T cell receptor Vβ repertoire gaps, among other effects [19, 21–32]. Recovery of immune function

Received 7 February 2000; revised 20 April 2000; electronically published 17 August 2000.


Informed consent was obtained from all participants in this study. Human experimentation guidelines of the US Department of Health and Human Services and of the authors’ institutions were followed.

Financial support: Campbell Foundation; John M. Lloyd Foundation; Philadelphia Foundation (Robert I. Jacobs Fund); Mrs. M. Stengel-Miller; AIDS funds from the Commonwealth of Pennsylvania; Wistar Institute’s HIV-1 Partnership Program for Basic Research; City of Philadelphia AIDS Activities Coordinating Office (clinical services); Tri-Institutional MD-PhD Program, GM 0773, and F31 GM20068 (G.M.O.); Hellmuth Hertz Foundation (J.S.); National Institutes of Health (AI-44595 to D.F.N. and RR-00040 to R.G.); Benjamin and Mary Siddons Measey Training Fellowship for Clinicians (Wistar Institute; to R.G.).

a E.P. and G.M.O. contributed equally to this work.

Reprints or correspondence: Dr. Luis J. Montaner, Wistar Institute, 3601 Spruce St., Philadelphia, PA 10104 (montaner@wistar.upenn.edu).

The Journal of Infectious Diseases 2000;182:766–75
© 2000 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2000/18203-01602.00
following HAART in chronically infected subjects has renewed the interest in augmenting anti-HIV-1 immune responsiveness in chronically infected persons. Increasing cell-mediated immunity against HIV-1 would be expected to delay disease progression and increase the efficacy of treatment by complementing antiretroviral therapy-mediated suppression with immune-mediated control. Strategies currently under study for boosting HIV-1–specific immune response include use of immune modulators to increase T cell and NK cell responsiveness, nonspecific interleukin-2 stimulation, and therapeutic vaccines based on heterologous HIV-1 antigens [33–35]. Obstacles to these approaches include the need to select adequate immunogens for therapeutic vaccines that are relevant to the host’s viral quasispecies, the need to sustain long-lasting T cell–mediated responses, and the toxicities of the immune modulators. Although prolonged HAART can have a positive effect by decreasing viral replication, it can also have a negative effect by decreasing HIV-1–specific cellular immune responses [16, 36–38].

The association between cellular immune responses against HIV-1 antigens and temporary suppression of HIV-1 replication in the absence of therapy has been recently documented in acutely infected subjects who were treated within 120 days of infection and then interrupted their treatment temporarily [39, 40]. Importantly, an association of viral replication with increased CD8 cell–mediated cellular immune responses following temporary drug discontinuation was observed [39]. This observation, along with the observation of preserved and enhanced CD4 T cell responses following suppressive therapy in newly infected subjects [19], has generated the hypothesis that periods of treatment interruption followed by reinitiated therapy in acutely infected persons may preserve and boost HIV-1–specific cellular immune responses. It has remained undetermined whether these observations of enhanced immune responses are applicable to chronically infected persons in light of their longer duration of immune dysfunction, CD4 T cell loss, and viral replication.

We undertook an observational study to determine if sustained HAART-mediated virus suppression followed by temporary therapeutic interruption could result in an enhancement of HIV-1–specific CD4 and CD8 T cell responses in chronically infected persons.

**Methods**

**Study subjects and samples.** We recruited 10 HIV-1–infected persons by word of mouth in a community-based HIV treatment center in Philadelphia. Five subjects without active intercurrent coinfections were identified: 1 had decided to interrupt antiretroviral therapy because of drug intolerance and 4 because of personal choice. Five control subjects who were not receiving antiretroviral therapy were also identified (table 1). Inclusion criteria for the interruption group were baseline CD4 cell percentage of >10% and >6 months of therapy-mediated virus suppression to <400 copies/mL before monitored therapy interruption. One subject in the interruption group (C-03) was recruited during therapy interruption after a period of 16 months of virus suppression. Inclusion criteria for the control group were a baseline CD4 T cell percentage of >10% and >4 months not receiving antiretroviral therapy at time of enrollment. All subjects were scheduled for monitoring of plasma HIV-1 RNA, CD4 T cell percentage, and immunologic parameters every 14 days. Virus load and CD4 T cell count were determined by Quest Diagnostics (Horsham, PA). Under institutional review board guidelines, all clinical results were provided to the subjects’ primary care providers in real time. Clinical management was solely the purview of the subjects’ providers and independent of this study.

**Anti-HIV-1–specific CD4 T helper activity.** In accordance with

### Table 1. Entry information for human immunodeficiency virus type 1 (HIV-1)–infected subjects, CD4 cell percentage, monitored therapy interruptions, and total follow-up.

<table>
<thead>
<tr>
<th>Group, subject</th>
<th>Sex</th>
<th>Age, years</th>
<th>HIV-1 RNA copies/mL</th>
<th>CD4 cells at baseline, %</th>
<th>Interruption period, days</th>
<th>CD4 cells at peak virus load, %</th>
<th>Total follow-up, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-03 Experimental</td>
<td>M</td>
<td>48</td>
<td>&lt;50a</td>
<td>11.8</td>
<td>61</td>
<td>8.4</td>
<td>260</td>
</tr>
<tr>
<td>C-11</td>
<td>M</td>
<td>47</td>
<td>&lt;50</td>
<td>28.0</td>
<td>54</td>
<td>18.0</td>
<td>181</td>
</tr>
<tr>
<td>C-13</td>
<td>M</td>
<td>43</td>
<td>&lt;40</td>
<td>23.7</td>
<td>46</td>
<td>25.0</td>
<td>212</td>
</tr>
<tr>
<td>C-15</td>
<td>M</td>
<td>43</td>
<td>&lt;50</td>
<td>24.3</td>
<td>72b</td>
<td>24.4</td>
<td>176</td>
</tr>
<tr>
<td>C-18</td>
<td>M</td>
<td>34</td>
<td>&lt;50a</td>
<td>27.0</td>
<td>56</td>
<td>22.0</td>
<td>176</td>
</tr>
<tr>
<td>C-05 Control</td>
<td>F</td>
<td>29</td>
<td>100,000</td>
<td>22.0</td>
<td>118b</td>
<td>19.0</td>
<td>118</td>
</tr>
<tr>
<td>C-07</td>
<td>M</td>
<td>37</td>
<td>66,700</td>
<td>22.0</td>
<td>56</td>
<td>18.0</td>
<td>181</td>
</tr>
<tr>
<td>C-09</td>
<td>M</td>
<td>30</td>
<td>12,000</td>
<td>22.0</td>
<td>56</td>
<td>18.0</td>
<td>181</td>
</tr>
<tr>
<td>C-12</td>
<td>F</td>
<td>37</td>
<td>7320</td>
<td>22.0</td>
<td>118b</td>
<td>19.0</td>
<td>118</td>
</tr>
<tr>
<td>C-17</td>
<td>M</td>
<td>53</td>
<td>53,900</td>
<td>22.0</td>
<td>118b</td>
<td>19.0</td>
<td>118</td>
</tr>
</tbody>
</table>

**NOTE:** Subject C-13 underwent 2 monitored therapy interruptions. Subject C-03 was undergoing interruption when recruited into the study; HIV-1 RNA level reflects baseline before subsequent, monitored interruption (see figure 2 for complete follow-up).

- a Subject reports instances of unmonitored therapy interruption.
- b Subject continues to receive therapy at end of follow-up for analysis.
optimal conditions for lymphoproliferative response analysis [41], peripheral blood mononuclear cells (PBMC) were isolated and used the same day of blood collection. PBMC were isolated by standard Ficoll-Paque (Pharmacia, Piscataway, NJ) density gradient centrifugation, washed 3 times in sterile 1× PBS, resuspended in RPMI 1640 (Cellgro, Herndon, VA) supplemented with 10% fetal bovine serum (Cansera, Ontario, Canada), 100 U/mL penicillin, 100 µg/mL streptomycin (Life Technologies, Grand Island, NY), and 2 mM glutamine (Life Technologies), and cultured in 96-well Falcon U-bottom plates (Becton Dickinson, Franklin Lakes, NJ) at 250,000 cells/well. Each sample was divided into 9 groups of 6 wells each to include 1 unstimulated control and 8 stimulated conditions as follows: 6 groups stimulated with insect cell–baculovirus recombinant HIV-1 antigens (HIV-1 p24 core protein including additional amino acids of the C-terminus of p17 and N-terminus of p15 with molecular weight of 35,000 on SDS/PAGE [Protein Sciences, Meriden, CT], HIV-1 LAV gp160 fully glycosylated with molecular weight of 160,000 on SDS/PAGE [Protein Sciences]), 1 positive control (phytohemagglutinin [5 µg/mL; Sigma, St. Louis]), and 1 negative control (keyhole limpet hemocyanin [5 µg/mL; Sigma] or a negative protein control provided by the manufacturers of recombinant viral antigens [5 µg/mL; Protein Sciences]). HIV-1–specific stimulations were done at 0.1, 0.5, and 5 µg/mL.

After 5 days in culture, all groups were pulsed for 18 h with tritiated thymidine (1 µCi/well; Amersham, Arlington Heights, IL). Nuclei were harvested by lysis of PBMC in each well in 70% ethanol and collected onto glass fiber filter paper (Packard, Meriden, CT) by use of an automatic multiwell harvester (Gast, Benton Harbor, MI). Radioactivity in the filter paper was quantified as cpm (antigen-stimulated cpm – unstimulated mean cpm) and as stimulation index (SI; antigen-stimulated mean cpm/unstimulated mean cpm). We defined a positive response as an SI of >3 because an analysis of >800 lymphoproliferative responses showed that a magnitude response of >2 represented a significant antigen-specific difference between unstimulated and antigen-stimulated controls in our assay. For data presentation, we elected to show both cpm to illustrate untransformed raw data and SI.

**Anti-HIV-1–specific CD8 T cell activity.** Anti-HIV-1–specific CD8 T cell activity was quantified by using ELISPOT to measure release of interferon-γ (IFN-γ) as described [39, 42]. Briefly, 96-well filtration plates (Millipore, Bedford, MA) were coated overnight at 4°C with 5 µg/mL primary anti-IFN-γ monoclonal antibody (Mabtech, Stockholm). The antibody-coated plates were washed 4 times with 1× PBS and blocked with RPMI containing 5% pooled human serum for 1 h. Uninfected and infected (recombinant vaccinia virus expressing either Gag, Env, Pol, or Nef or no HIV-1 antigen as control) PBMC were added to the wells and incubated overnight. Wells were washed 4 times with PBS followed by a 2-h incubation with the secondary antibody at 1 µg/mL. Plates were washed 4 times in PBS with 0.1% Tween 20. Avidin-bound horseradish peroxidase H was added to the wells for 1 h at room temperature; the plates were washed 4 times with 0.1% Tween 20 and then developed with diaminobenzidine tetrahydrochloride. The spots were counted with a stereomicroscope, and vaccinia virus control signals for each donor were subtracted from each corresponding HIV-specific response. Only spots with a fuzzy border and a brown color were counted. Results were expressed as spot-forming cells (SFC)10⁶ PBMC. From our previous studies of HIV-1–infected subjects we have established this response to be CD8 T cell mediated and have proposed a descriptive range for the strength of cytotoxic T lymphocyte responses: low, 10–200 SFC/10⁶ PBMC; moderate, 201–500 SFC/10⁶ PBMC; high, >501 SFC/10⁶ PBMC [39, 42].

**Flow cytometry analysis.** Flow cytometry analysis was done at entry of each patient in the study. Whole blood was stained with 5 staining combinations by use of directly conjugated antibodies. Combinations of anti–cell surface antigen antibodies used were as follows: CD4/CD28/HLA-DR, CD8/CD28/HLA-DR, CD4/CD45RA/CD45RO, CD4/TNF-RII, and CD3/CD95. The fluorochrome-conjugated monoclonal antibodies CD3-PE (phycoerythrin), CD4-PE, CD8-PE, CD28-FITC (fluorescein isothiocyanate), CD45RO-FITC, and CD95-FITC were purchased from Pharmingen (San Diego) and CD4-FITC, CD8-TC, CD45RA-TC, and HLA-DR-TC were purchased from CalTag (Burlingame, CA). TNF-RII-PE was obtained from R&D Systems (Minneapolis). The isotype-matched controls IgG1-FITC, IgG1-PE, IgG2a-FITC, IgG2a-PE, IgG2a-TC, and IgG2b-TC were purchased from Pharmingen. Briefly, 75 µL of whole blood was incubated with 7.5 µL of fluorescence activated cell sorter (FACS) blocking buffer (1× PBS, 0.2% bovine serum albumin, 10% mouse serum, 0.1% sodium azide) for 15 min at room temperature and stained with the appropriate monoclonal antibody for 50 min at room temperature. The cells were then lysed with lysis buffer (FACS Lys, Becton Dickinson Immunocytometry Systems, San Jose, CA) for 10 min at room temperature. After being washed twice with FACS washing buffer (1× PBS, 2.5% heat-inactivated fetal bovine serum, 0.1% bovine serum albumin, 0.02% NaN3), the cell pellets were resuspended in 500 µL of FACS washing buffer and analyzed on a Becton Dickinson FACSCalibur flow cytometer with use of the CellQuest software package for acquisition and analysis. Gating of lymphocytes was done during acquisition of 10,000 events for each condition. Analysis on lymphocytes and thresholds were set according to isotype-matched negative controls. Results were expressed as percentage positive.

**Statistics.** The data were described with means, medians, SDs, and ranges for both groups. Virus load data were transformed to the log scale for the purpose of analysis. Differences between specific time points within groups or between groups for CD4 T cell percentages and flow cytometry data analysis were tested by use of the Wilcoxon signed rank and Wilcoxon rank sum tests, because the data were not normally distributed. We used regression analysis to estimate the slope of each person’s lymphoproliferative and SFC activity. We used generalized estimating equations (GEEs) to accommodate the correlated nature of the lymphoproliferative and SFC data (i.e., multiple observations over time per person) [43]. Analyses were done with Stata 5.0 (Stata, College Station, TX) and JMP 3.2 (SAS Institute, Cary, NC). All P values are two-sided.

**Results**

**Therapy interruption and changes in virus load and CD4 T cell percentage.** Five experimental subjects who planned a
therapy interruption (C-03, -11, -13, -15, and -18) were identified. They had received a median of 24 months of antiretroviral therapy preceding this study with a history of plasma virus load of <400 copies/mL for the duration of this period. Two subjects in the experimental group had a history of prior treatment interruption. Subject C-03 had a treatment interruption lasting 91 days (recruited for follow-up at day 56), followed by a resumption of antiretroviral therapy before a second therapy interruption 78 days later. This second time period is presented as the baseline time point in table 1. Subject C-18 reported several unmonitored treatment interruptions during the 29 months before enrollment. The 5 control subjects, C-05, -07, -09, -12, and -17, all had a history of plasma virus loads >5000 copies/mL (median of 4 months).

All experimental subjects had virus loads of <50 copies/mL at the time of monitored interruption, with plasma virus load increasing above this level in 4 subjects within 2 weeks of stopping therapy (rate of virus load increase for the group: 0.045 log copies/day \(P < .001\); range, 0.005–0.08). The fifth subject, experimental subject C-03, did not experience plasma virus load of >50 copies/mL until 43 days after monitored therapy interruption, when a virus load of 10,100 copies/mL was measured. The rebound in virus was associated with an acute diagnosis of oral thrush. Although C-03 decided to reinitiate therapy at 14 days after this initial virus rebound, his virus load at that time (day of therapy reinitiation) had decreased by 80%. Overall, 4 of the 5 experimental subjects, C-03, -11, -13, and -15, reinitiated treatment after a median of 55 days of therapy interruption (table 1), with a median peak virus load during this period of 57,800 copies/mL. All 4 subjects (3 of 4 restarted the same HAART regimen) achieved a mean 98.86% suppression of virus load 21–33 days after reinitiating therapy, showing that although virus rebound was observed following therapy interruption, it remained highly responsive to reinitiation of therapy [44]. The remaining experimental subject, C-18, maintained a plasma virus load of <1080 copies/mL for the duration of follow-up in the absence of therapy reinitiation (i.e., 118 days).

Experimental subjects did not have significant differences in CD4 T cell percentages differences between time points, comparing baseline with the time point of peak virus load after interruption and with the final available time point after therapy reinitiation (Wilcoxon signed rank test; \(P > .05\); figures 1 and 2). However, decreases in CD4 T cell percentage did occur in specific patients, such as C-11, who experienced up to a 35.7% decline in CD4 T cell percent during the interruption period. No significant changes were observed for controls (Wilcoxon signed rank test; \(P > .05\)). CD4 T cell percentage data for 2 representative controls are shown in figure 3.

Continued follow-up of experimental subjects following reinitiated therapy included a subsequent monitored interruption in therapy by C-13 after achieving virus suppression to <50 copies/mL (figure 1; table 1). Surprisingly, this subject showed a prolonged period of virus suppression and a lower magnitude of virus rebound at the second interruption compared with the first. In addition, C-13 showed a virus response similar to that of C-03; both had a virus rebound that subsequently declined over time without a significant drop in CD4 T cell percentage during this period. Taken together, our observations after therapy reinitiation show that plasma virus was efficiently resuppressed, and no significant drops in CD4 T cell percentages were detected in these subjects as a group.

Baseline immunophenotype of T cell subsets. To compare the T cell immunophenotype of experimental subjects with that of controls, CD4 and CD8 T cell subsets were evaluated for their expression of a set of cell surface markers associated with disease progression, immune activation, and function. The history of virus suppression in the experimental group was associated with a significant increase in baseline CD4+ CD28+ HLA-DR (Wilcoxon rank sum test; \(P = .047\)) and decreases in CD8+ CD28+ HLA-DR (Wilcoxon rank sum test; \(P = .016\)) and CD3+ CD95 (Wilcoxon rank sum test; \(P = .047\)) T cell subsets compared with controls. Among individual markers analyzed in the lymphocyte gate, only HLA-DR was found to be significantly decreased in the experimental group (Wilcoxon rank sum test; \(P = .036\)). These differences between suppressed and viremic patients are consistent with described T cell phenotypes following prolonged virus suppression [25]. However, no significant differences in total CD4, CD8, or CD4+ CD45RA+ T cell subsets were observed between groups, demonstrating that these 2 groups were not dissimilar in markers of disease progression.

Virus rebound and anti-HIV-1 CD4 T cell proliferative responses. HIV-1–specific T helper activity was assessed by measuring lymphoproliferative responses against HIV-1 antigens p24 and gp160 over 3 antigen concentrations. In spite of fluctuations in \(\Delta^{cpm}\) values in the experimental subjects after treatment interruption (figure 1; C-11 vs. C-13), the use of GEEs [43, 45, 46] for the longitudinal analysis of HIV-1 p24 SI in the experimental group showed a significant increase over the first 30 days of treatment interruption (1.82 SI/week; \(P = .001\)), in contrast to controls (0.042 SI/week; \(P = .90\)). Furthermore, comparison of the slopes of the SI response for HIV-1 p24 in the experimental group versus controls showed a significant difference over this period of follow-up (GEEs, \(P = .014\); figures 1 and 2). Analysis of HIV-1 p24 SI responses from baseline to 21–33 days of therapy reinitiation (time to 98.86% virus suppression) showed an increase for all experimental subjects (median increase, 2.7 SI). Differences in gp160 antigen response over these time periods were not statistically significant. Consistent with enhanced T helper responses against p24 in association with therapy interruption, a subsequent monitored interruption in therapy in C-13 showed a further increase in response when comparing p24 SI (or lymphoproliferative responses) with respective responses at time of study entry or immediately before the second interruption (figure 1). Independent confirmation of the significance of the
Figure 1. Longitudinal analysis of virologic and immunologic parameters in chronically human immunodeficiency virus type 1 (HIV-1)-infected patients undergoing antiretroviral treatment interruption without history of therapy interruption. Longitudinal profiles for subjects C-11, C-13, and C-15 are shown; bars on top illustrate period without therapy from time of interruption (baseline) and subsequent therapy reinitiation. Entry information for all patients is given in table 1. Top to bottom: plasma HIV-1 RNA; CD4 T cell %; anti-HIV-1 T helper responses against HIV-1 p24 and HIV-1 gp160 antigens (5 µg/mL) as stimulation index (SI; gray area defines negative response range of <3 SI) and as raw data ([counts per minute] = antigen-stimulated mean cpm [6 wells] − unstimulated mean cpm [6 wells]); and anti–HIV-1 CD8 cell-mediated interferon-γ–secreting cells against HIV-1 Gag, Env, Pol, and Nef, shown as frequency of HIV-1–specific spot-forming cells (SFC)/10^6 peripheral blood mononuclear cells (PBMC; gray area defines negative response range at <10 SFC/10^6 PBMC).

A range of responses measured was obtained from two independent lymphoproliferative responses at lower concentrations of antigen, determined at each time point, indicating a dose response for each positive SI response (data not shown). In addition, the possibility that the increase in HIV-1 p24 SI in the experimental group could be due to a nonspecific decrease in baseline cpm (i.e., increase in SI due to a decrease in the denominator rather than an increase in the numerator) was ruled out by demonstrating a lack of downward trend in baseline cpm over times tested (GEEs, P > .05).

Analysis of p24 SI responses from time of reinitiated therapy to 95 days of subsequent follow-up (maximum period in common for all subjects) showed no further increase. In addition, no significant change in the magnitude of the p24 SI was found by GEE analysis (P > .05) over this period. The remaining subject who elected not to reinitiate therapy (C-18) during follow-up maintained positive SI responses against p24 in the presence of sustained low-level viral replication (figure 2).

Controls followed for an equal period showed a consistent absence of any sustained positive SI response against either p24 or gp160, suggesting an absence of T helper function in these subjects (figure 3). Taken together, our lymphoproliferative results demonstrate an increase in HIV-1–specific CD4 T helper responses in chronically infected persons in association with virus rebound following therapy interruption.

Virus rebound and antiviral IFN-γ–secreting CD8 T cell responses. HIV-1–specific CD8 T cell responses were measured as SFC by ELISpot against HIV-1IIIB Env, Gag, Pol, and Nef proteins at all patient contact times [42]. The association between rising HIV-1–specific SFC and virus load was apparent in subjects without a history of therapy interruption (C-11, C-13, C-15). These subjects showed an over-
all low mean baseline SFC frequency against all 4 HIV-1 antigens tested combined with a virus load rebound of >10^4 copies/mL (figure 1). However, subjects with a history of previous therapy interruption, such as C-03 and C-18, showed high baseline SFC frequencies that persisted for the duration of the study period (figure 2). In contrast to the lymphoproliferative responses described above, CD8 T cell responses were present at high frequency throughout the study period in the control subjects (see representative controls in figure 3).

Analysis of changes from baseline in the magnitude of SFC responses per day during the period of therapy interruption showed a significant increase in the response to HIV-1 Env (117.6 SFC/week; GEEs, \( P = .016 \)) and Nef (47.6 SFC/week; GEEs, \( P = .03 \)). As for lymphoproliferative responses, analysis at 21–33 days of therapy reinitiation (time to 98.86% virus suppression for C-03, C-11, C-13, and C-15) showed a significant increase in the magnitude of antiviral IFN-\( \gamma \)-secreting CD8 T cell responses from baseline. Specifically, a significant
Figure 3. Longitudinal analysis of virologic and immunologic parameters in chronically human immunodeficiency virus type 1 (HIV-1)-infected patients in absence of antiretroviral therapy. Representative longitudinal profiles for control subjects C-5 and C-12 are shown. Entry information for both patients is given in Table 1. Top to bottom: plasma HIV-1 RNA; CD4 T cell %; anti-HIV-1 T helper responses against HIV-1 p24 and HIV-1 gp160 antigens (5 μg/mL) as stimulation index (SI; gray area defines negative response range of <3 SI) and as raw data (Δcpm [counts per minute] = antigen-stimulated mean cpm [6 wells] - unstimulated mean cpm [6 wells]); and anti-HIV-1 CD8 cell-mediated interferon-γ-secreting cells against HIV-1 Gag, Env, Pol, and Nef, shown as frequency of HIV-1-specific spot-forming cells (SFC)/10^6 peripheral blood mononuclear cells (PBMC; gray area defines negative response range at <10 SFC/10^6 PBMC).

Analysis of CD8 T cell responses from the time of therapy initiation to 95 days of subsequent follow-up (maximum period in common for all subjects) showed a trend toward a decrease in the magnitude of responses against HIV-1 Env (102.9 SFC/week; GEEs, P < .001) and Nef (42 SFC/week; GEEs, P = .008) was confirmed, along with a significant increase against HIV-1 Pol (58.1 SFC/week; GEEs, P = .002). No significant differences between comparable time points were observed among controls. Comparison of SFC response slopes for each antigen from baseline to 21–33 days of reinitiated therapy in experimental subjects versus a comparable time in controls showed a significant difference in response to HIV-1 Env in the experimental group in relation to controls (74.9 SFC/week; GEEs, P = .004). Figure 4 shows all subjects' longitudinal raw data used in the previous analysis of HIV-1 Env SFC/10^6 PBMC (C-18 was excluded because of lack of reinitiated therapy). No other antigen response slope showed a significant change, when compared between groups.

Discussion

Our data support the hypothesis that a time-limited episode of increased HIV-1 replication could result in significant increases in anti-HIV-1-specific CD4 cell–mediated lymphoproliferative and CD8 cell–mediated IFN-γ-secreting T cell responses in chronically infected persons with prior sustained virus suppression under treatment for >6 months. Although a sustained low level of viral replication is present in subjects...
receiving HAART [47, 48]. HIV-1–specific responses are generally weak, intermittent, or both. Our results suggest that the magnitude and duration of viral replication is an important factor for the expansion of immune-mediated responses against endogenous viral antigens.

Our observational data suggest that an empirically defined period of 55 days of therapy interruption in patients is associated with a median peak virus load of 57,800 copies/mL and an increase of anti–HIV-1 lymphoproliferative responses to Gag antigens (figures 1 and 2). However, chronically viremic controls indicate that these responses can be eventually lost in patients sustaining high levels of viral replication (median baseline virus load, 53,000 copies/mL) (figure 3). As suggested by studies of early infection [19], therapy reinitiation may be a critical factor in preserving de novo or boosted CD4 T cell responses against HIV-1. However, further analysis of enhanced lymphoproliferative responses during reinitiated suppressive therapy will be needed to establish the half-life of these responses, because prolonged virus suppression has also been associated with a decrease in HIV-specific T helper responses [16, 38]. It also remains to be determined whether our data indicate that periods of virus breakthrough induced by interleukin-2 immunotherapy, prophylactic vaccinations, or coinfections can result in enhanced HIV-1–specific lymphoproliferative responses.

CD8 T cell–mediated responses were also observed to increase during therapy interruption and reinitiation of therapy, suggesting a positive effect of viral replication and acute suppression on the expansion of CD8 T cell–mediated responses and or a redistribution of expanded antiviral CD8 T cells into circulation (figures 1 and 2). In either case, these observations, together with the apparent decrease in enhanced responses against HIV-1 Env in the experimental group over 95 days of reinitiated therapy, support the concept that HIV-1 replication may serve as the mechanism for maintaining HIV-1–specific CD8 T cell–mediated responses [37, 49, 50]. However, the level of CD8 T cell anti–HIV-1 responses present in controls demonstrates that these responses alone may be insufficient to completely suppress viral replication and point to the difference in T helper activity between both groups as a potential critical component in enhancing functional CD8 cytotoxic responses, as has been suggested by others [32, 51] (figure 3). The absence of anti-Env CD4 cell proliferative responses in the presence of increased anti-Env CD8 cell responses remains unexplained by our data. Further analysis will need to determine if CD4 cell lymphoproliferative responses against envelope antigens are not effectively induced following treatment interruption in chronic infection or whether the antigen used for in vitro restimulation is not representative of the antigens present on primary isolates in vivo.

Importantly, we show that HIV-1–specific cell-mediated responses could be increased in chronically suppressed patients following a period of high viral replication in the absence of a significant decline in CD4 T cell percentages during the periods studied. Indeed, long-term follow-up of the measurable decreases in CD4 T cell percentage observed at peak virus load during therapy interruption (table 1) suggests that these decreases may be reversible, as is seen with the initiation of suppressive antiretroviral therapy [52–54] (e.g., see C-11 in figure 1).

A correlation between magnitude of lymphoproliferative and SFC responses with suppression of viral replication cannot be made on the basis of this study. However, it was observed that high levels of lymphoproliferative and SFC responses at the time of interruption were present in conjunction with a lower level of virus rebound in subjects C-03, C-13 (second interruption), and C-18. Detection of plasma viremia was delayed or maintained at a lower level in all of these subjects during the
first 5 weeks of interruption (≤50–1080 HIV-1 RNA copies/mL). In contrast, the other interrupting patients, including C-13 during the first interruption period, lacked similar magnitude of anti-HIV-1–specific cell-mediated responses at baseline and rebounded to higher virus loads (15,600–632,000 HIV-1 RNA copies/mL) over the same period. Additional studies will be needed to determine whether levels of antiviral responses acquired previous to an interruption in therapy (i.e., previous interruptions) may affect the time and magnitude of virus rebound, as suggested by our data.

Most important, our observational study advances the rationale for further investigation of structured therapy interruptions in chronically infected persons as a potential mechanism for augmenting anti–HIV-1–specific immunity. Indeed, periodic stimulation of the host’s immune system with viral antigen may be a necessary component of sustaining CD4 and CD8 T cell cytotoxic memory responses able to delay disease progression and limit breakthrough of virus on noncompliance with therapy or its discontinuation. If beneficial, it will also be important to determine if this intervention is most efficacious alone or in combination with other immune-targeted strategies (e.g., interleukin-2, therapeutic vaccines) [33–35] to complement and extend the durability of antiretroviral chemotherapy. Of course, safety concerns must be carefully assessed in relation to any therapy interruption, given our understanding of the mechanism underlying viral resistance and the potential for loss of CD4 T cells. However, given the current limitations of antiretroviral therapy, we do not believe that the potential risk of this intervention inherently outweighs the potential benefit and believe that further study of strategic therapy interruption is warranted.

Acknowledgments

We thank the HIV-1–infected subjects; providers of clinical care (S. Allen, M. Vandenberg, R. Jones, J. Stewart, H. Kwakwa, G. Stewart, and J. Ondercin); A. Green for technical assistance and patient coordination; D. Davis, R. Smith, P. Lloynd, P. Cooper, and R. Anthony for study assistance; the board and staff of Philadelphia Field Initiating Group of HIV-1 Trials; and D. Herlyn for critical manuscript review.

References


49. Evans TG, Bonnez W, Souciar HR, Fitzgerald T, Gibbons DC, Reichman RC. Highly active antiretroviral therapy results in a decrease in CD8+ T cell activation and preferential reconstitution of the peripheral CD4+ T cell population with memory rather than naive cells. Antiviral Res 1998;39:163–73.